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Cholesterol lowering efficacy of plant stanol ester in a new type of product matrix, a chewable dietary supplement



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ABSTRACT

Low-density lipoprotein (LDL) cholesterol lowering efficacy of a new type of chewable plant stanol ester food supplement was evaluated in a randomized, double-blind, controlled four-week intervention. The participants (LDL cholesterol > 3 mmol/L) consumed four supplements daily with meals either with (n = 50) or without (n = 53) plant stanol esters. Plant stanol ester supplement (2 g/d plant stanols) lowered LDL cholesterol by 7.6%, serum cholesterol by 4.9%, and non-high density lipoprotein (HDL) cholesterol by 6.6% compared with controls (P < 0.003). HDL cholesterol or serum triacylglycerol concentrations were unchanged. The taste of the supplement was considered good/very good by 68% of the responders, and convenience to consume it was considered easy/very easy by 78% of the responders. No side effects were reported.

In conclusion, this new type of small-volume chewable plant stanol ester supplement lowered LDL cholesterol concentration in hypercholesterolemic subjects providing a convenient dietary tool to regulate circulating cholesterol levels.

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1. Introduction

Modification of diet is an essential approach for the management of elevated low density lipoprotein (LDL) cholesterol concentration either alone or alongside with lipid lowering medication. The efficacy of plant stanol esters administered in foods especially in spreads and yoghurt drinks is established to lower LDL cholesterol concentration by 9–10% at a daily dose of 2 g plant stanols (Demonty et al., 2009; Musa-Veloso, Poon, Elliot, & Chung, 2011; Ras, Geleijnse, & Trautwein, 2014). This amount of LDL cholesterol reduction is clinically relevant, since it is estimated that for every 1% reduction in LDL cholesterol concentration there is a corresponding 1% decrease in the risk of coronary artery disease (LaRosa, 2007).

There is a need for new dietary tools that are, in addition to having proven efficacy, convenient to use daily. Plant stanols inhibit cholesterol absorption by displacing cholesterol from mixed micelles in the proximal intestine so that less cholesterol is trans-

ported from the intestine to the liver (Armstrong & Carey, 1987; Ikeda, Tanaka, Sugano, Vahouny, & Gallo, 1988; Miettinen, Vuoristo, Nissinen, Järvinen, & Gylling, 2000; Racette et al., 2010). The whole process is complicated and vulnerable to disturbances. It is even possible that no LDL cholesterol lowering occurs (Davidson et al., 2001; Denke, 1995; Ottestad et al., 2013). Consequently, when plant stanol esters are added to a novel type of food matrix, even the structure and volume of the product matrix has an impact on the success of cholesterol absorption inhibition, and the functionality of new types of products should be confirmed with clinical studies. It is noteworthy that most of the studies included in the large meta-analyses and to which the LDL cholesterol lowering efficacy of plant stanol ester food products is based are performed with plant stanols/sterols added to solid foods or large-volume yoghurts and drinks. Regarding small-volume products, there is recent evidence that softgel capsules with added plant stanols/sterols have substantial variation in their LDL cholesterol lowering efficacy. In the five published studies, plant stanol/sterol softgel capsules had either no effect (Ottestad et al., 2013) or they reduced the placebo-corrected LDL cholesterol concentration from 4% to 9% (Maki et al., 2012; Maki et al., 2013; McKenney et al., 2014; Woodgate, Chan, & Conquer, 2006). The doses of added plant stanols or plant sterols did not explain the conflicting results,

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because the dose was even larger (2.0 g/day) in the negative than in the four positive studies (1.6 or 1.8 g/day).

In this study, a new type of plant stanol ester food supplement was developed. Its matrix is structurally completely different from commercially available plant stanol or plant sterol supplements such as softgel capsules or tablets (e.g., Maki et al., 2012; Maki et al., 2013; McKenney et al., 2014; Ottestad et al., 2013; Woodgate et al., 2006). This small-volume low-fat chewable and easy to swallow food supplement is based on emulsified plant stanol esters in a gelled water phase in order to ensure effective release of the emulsified plant stanol esters from the product matrix in the stomach. To this end, the aim of this study was to investigate the LDL cholesterol lowering efficacy of this new plant stanol ester dietary supplement in a four-week randomized, double-blind, controlled intervention in adult subjects with hypercholesterolemia.

2. Methods

2.1. Study population

A total of 131 volunteers were recruited from the Helsinki city area through advertisements in a local newspaper, on notice boards in Helsinki University Central Hospital, and also through contacting potentially eligible study participants from our earlier studies (Fig. 1). The study was conducted at Helsinki University Central Hospital, Helsinki, Finland. The inclusion criteria were: men and women over 18 years of age, LDL cholesterol concentration > 3 mmol/L, and body mass index (BMI) < 35 kg/m². Exclusion criteria were: kidney, liver, or thyroid malfunction, inflammatory

bowel disease, unstable chronic disease such as coronary heart disease or coronary procedure within six months, lipid-lowering medication, or the consumption of nutrient supplements interfering with serum cholesterol level (red rice or berberine), gravidity or breast feeding, allergy or hypersensitivity to any component of the test product, alcohol consumption > 45 g absolute alcohol/day, or participating simultaneously in another clinical trial. If the subjects had used plant stanol/sterol products, they could be included in the study after a two weeks' wash out period.

All subjects gave their written informed consent prior to the inclusion in the study. The study was performed according to the principles of the 1964 Declaration of Helsinki and its later amendments. The Ethics Committee of the Department of Medicine, Hospital District of Helsinki and Uusimaa had approved the study protocol. The trial was registered at ClinicalTrials.gov (NCT0221297).

2.2. Study design

The study was a randomized, controlled, double-blind and parallel clinical trial with an intervention phase of four weeks. The subjects were divided into plant stanol and control groups. Randomization was executed in blocks of 10 subjects using an Internet-based program by the manufacturer of the supplement who also kept the code sealed until the last study visit. Enrolment of the participants was performed continuously according to a randomization list with three digit random numbers thus both the researchers and study participants were blind to the study code. The subjects attended a screening visit before randomization and two visits after enrolment, one at baseline and one at the end of the intervention. During the visits, fasting blood samples were

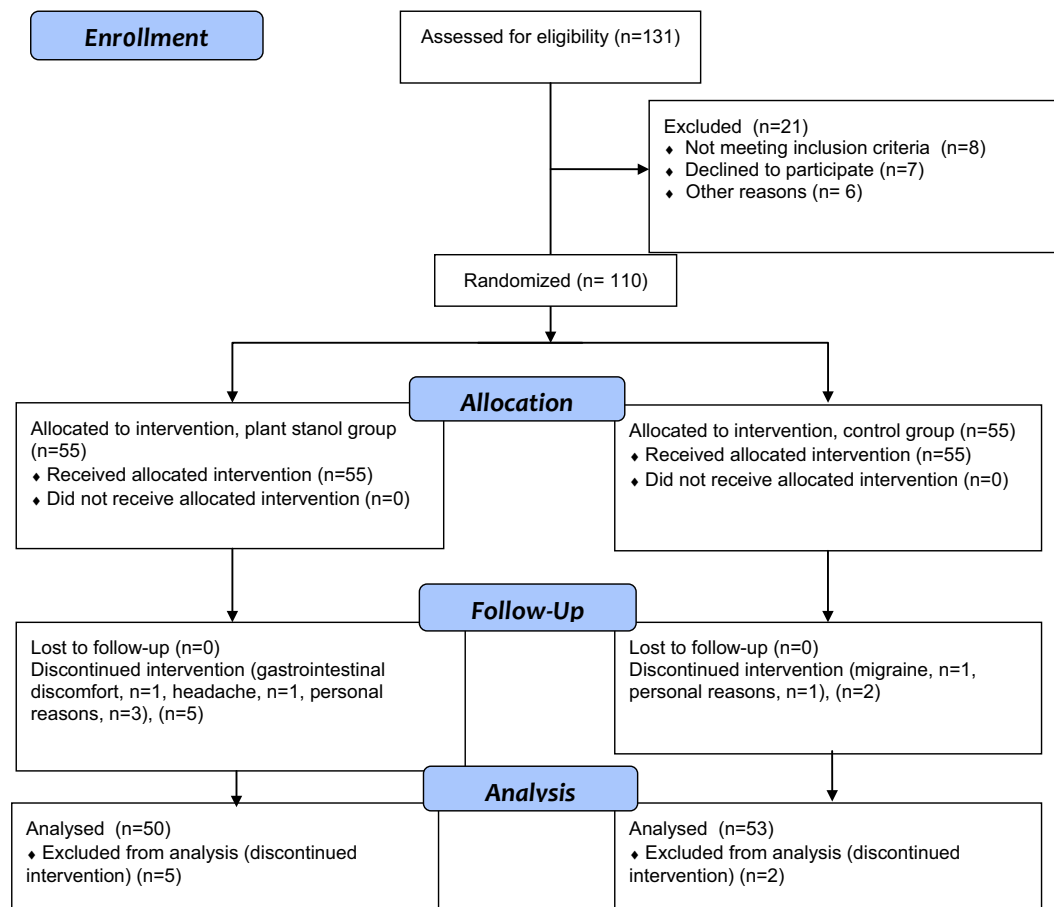


Fig. 1. Flow chart of the study.

taken, and weight (all visits), height (at screening), waist circumference (at baseline), and blood pressure (at baseline) were measured. The subjects were requested not to alter their lifestyle habits including diet and exercise during the study. Possible concomitant medication should have remained unchanged for 1 month before the study and, if possible, during the study.

The primary outcome was the proportional change in LDL cholesterol concentration (end of intervention minus baseline) between the study groups in subjects completing the study. Secondary outcomes were the proportional change in the serum total cholesterol, non-high density lipoprotein (HDL) cholesterol, HDL cholesterol, and serum triacylglycerol concentrations between the study groups in subjects completing the study.

2.3. Test products

The participants were advised to consume four pieces of the test supplements (Raisio Nutrition Ltd., Raisio, Finland) daily with meals in two daily lots for four weeks. The supplements were chewable in consistency and did not require water for consumption. The daily dose of plant stanols was 2 g. The supplement was sugar free and sweetened with xylitol and flavored with lemon and lime. The nutritional composition of the supplements is presented in Table 1. The supplements were packed in aluminium blisters that could be stored at room temperature. The supplements with and without plant stanol ester were packed in identical blisters and cardboard packages, which were labeled with individual three digit random numbers according to the randomization list. Thus, in the parallel study design, the intervention and placebo product could not be identified by the participants or the researchers executing the study, despite the slight difference in consistency of the two test products.

2.4. Compliance

Participants kept a diary daily on the intake of the study products and on any potential symptoms experienced during the consumption of the products. The participants were asked to return all blisters at the last study visit. The compliance was calculated as a proportion of the number of the consumed supplements (empty blisters) from the number of the prescribed supplements for the four-week intervention period. The participants filled in a questionnaire relating to sensory properties and experiences on using the supplement.

2.5. Dietary intake

Dietary intake was assessed prior to the baseline and the last study visit with 3-day food diaries covering 2 day during the week and 1 day on the weekend. The mean daily intakes of energy and nutrients were calculated using computerized dietary analysis program (AivoDiet, Aivo Finland Ltd., Turku, Finland).

Table 1
Nutritional composition of the intervention supplements.

Nutrition values 4 pieces (6.7 g), daily portion	Plant stanol ester supplement	Control supplement
Energy (kJ)	80	100
Total fat (g)	1.5	1.6
of which - saturated (g)	0.1	0.1
Total carbohydrates (g)	1.6	3.1
of which - sugars (g)	0.0	0.0
- polyols (g)	1.3	3.1
Total protein (g)	0.3	0.4
Plant stanols (g)	2.0	0.0

2.6. Blood sampling and analyses

Fasting blood samples were drawn from the antecubital vein after 12-hours' fasting. The subjects were advised to avoid heavy exercise and not to drink alcohol on the preceding day. Serum total, HDL cholesterol, serum triacylglycerols, and plasma glucose were determined enzymatically using automated analyzers with standardized methods at the certified Central Laboratory of Helsinki University Hospital (HUSLAB). LDL cholesterol was calculated using Friedewald's equation, and the results were checked with the enzymatic analysis at HUSLAB. Non-HDL cholesterol was calculated as follows: non-HDL cholesterol = total cholesterol – HDL cholesterol.

2.7. Statistical analyses

Statistical analyses were performed with SPSS for Windows 19.0 statistics program (SPSS, Chicago, IL, USA). The number of subjects recruited was based on a power analysis to detect a 10% difference in LDL cholesterol response between the study groups with an α level of 0.05 and with statistical power of 0.80. The normality and homogeneity of variance assumptions were checked before further analyses, and all variables were normally distributed. The comparisons between the plant stanol and control groups were tested using independent samples *t*-test. Non-continuous variables were tested with chi-square or Fisher's exact test. A *P*-value of <0.05 was considered statistically significant. The results are given as mean \pm SE.

3. Results

3.1. Demographics and clinical characteristics at baseline and after intervention

Of the 110 subjects randomized to the intervention, five subjects dropped out from the plant stanol group, one because of headache and one for gastrointestinal discomfort, and three for personal reasons (Fig. 1). Two subjects dropped out from the control group, one because of migraine and one for personal reasons. Accordingly, 103 subjects, 50 in the plant stanol and 53 in the control group completed the study and were included in the analyses.

The main clinical characteristics of the study population are demonstrated in Table 2. Age varied from 27 to 84 years with a mean value of 60 ± 1 (SE) years in the whole study population, and LDL cholesterol concentration varied from 3.08 to 6.69 mmol/L with a mean value of 4.1 ± 0.1 mmol/L, respectively. Seven subjects had elevated serum triacylglycerol concentration varying from 2.01 to 3.29 mmol/L. All subjects with a history of hypothyreosis were on adequate medication. The two subjects with type 2 diabetes in the control group were on oral hypoglycemic treatment with good glycemic control and without complications. The gender distribution, demographics, clinical characteristics including prevalence of diseases and different types of medication, and serum and lipoprotein cholesterol concentrations at baseline did not differ between the groups (Tables 2 and 3). The only exceptions were the slightly higher plasma glucose concentration in the control than in the plant stanol group because of the two diabetics present in the control group, and serum triacylglycerol concentration was higher in the control than in the plant stanol group. The mean plasma glucose and serum triacylglycerol concentrations were, however, within the general reference values in both groups (plasma glucose < 6.0 mmol/L, serum triacylglycerol \leq 1.70 mmol/L). Moreover, the higher plasma glucose and serum triacylglycerol

Table 2
Baseline characteristics.

Variables	Plant stanol ester group (n = 50)		Control group (n = 53)		p ^{a,b}
Gender (M/F), n	11/39		15/38		0.462
Age (y)	59.6	(±1.4)	60.1	(±1.4)	0.812
Weight (kg)	75.7	(±1.8)	73.4	(±1.6)	0.329
Body mass index (kg/m ²)	26.4	(±0.5)	26.0	(±0.5)	0.607
Waist circumference (cm)	93.6	(±1.4)	92.5	(±1.4)	0.567
Plasma glucose (mmol/L)	5.3	(±0.6)	5.7	(±0.9)	0.020
<i>Diseases, n</i>					
Hypertension	9		15		0.216
Hypothyreosis	5		10		0.202
Asthma bronchiale	2		8		0.057
Diabetes mellitus, type 2	0		2		0.165
Atrial fibrillation	2		0		0.141
<i>Medication, n</i>					
Angiotensin receptor blockers or angiotensin converting enzyme inhibitors	6		7		0.854
Diuretics	4		3		0.637
Beta blockers	1		6		0.060
Calcium channel blockers	1		4		0.190
Thyroxin	5		10		0.202
Contraceptives or hormone replacement therapy	5		4		0.660

Mean (± SE).

^a Independent samples *t*-test.^b chi-square or Fisher's exact test.**Table 3**
Serum and lipoprotein lipids (mmol/L) at baseline and after 4 weeks' intervention and change (mmol/L) from baseline.

Variables	Plant stanol ester group, n = 50		Control group, n = 53		Mean difference in response		p ^a
	Mean	(SE)	Mean	(SE)	Mean	(SE)	
<i>LDL cholesterol</i>							
Baseline	4.05	(±0.10)	3.99	(±0.11)			0.700
After intervention	3.85	(±0.09)	4.10	(±0.12)			0.096
Change (mmol/L)	−0.20	(±0.06)	+0.11	(±0.07)	−0.31	(±0.09)	0.001
<i>Serum total cholesterol</i>							
Baseline	6.34	(±0.11)	6.38	(±0.11)			0.794
After intervention	6.18	(±0.10)	6.54	(±0.13)			0.030
Change (mmol/L)	−0.16	(±0.07)	+0.16	(±0.07)	−0.32	(±0.10)	0.002
<i>Non-HDL cholesterol</i>							
Baseline	4.53	(±0.11)	4.62	(±0.13)			0.580
After intervention	4.35	(±0.10)	4.75	(±0.14)			0.024
Change (mmol/L)	−0.18	(±0.07)	+0.13	(±0.07)	−0.31	(±0.09)	0.001
<i>HDL cholesterol</i>							
Baseline	1.81	(±0.07)	1.76	(±0.06)			0.580
After intervention	1.83	(±0.07)	1.79	(±0.07)			0.703
Change (mmol/L)	+0.02	(±0.02)	+0.03	(±0.03)	−0.01	(±0.04)	0.706
<i>Serum triacylglycerols</i>							
Baseline	1.07	(±0.06)	1.40	(±0.13)			0.018
After intervention	1.11	(±0.07)	1.44	(±0.12)			0.018
Change (mmol/L)	+0.04	(±0.04)	+0.04	(±0.06)	0.002	(±0.07)	0.982

^a Between groups; independent samples *t*-test.

values in the control group did not affect the lipid results. There was no sex difference in demographics, clinical characteristics, or in serum and lipoprotein lipids in either of the study groups (NS for all, data not shown).

At the end of the intervention, weight of the subjects was unchanged (76.1 ± 1.8 kg, plant stanol ester group, and 73.5 ± 1.5 kg, control group, NS between the groups). The test products were well tolerated, and no side effects were reported. Based on calculating the returned supplements, 98% (74–107%) of the supplements were consumed by the participants. The majority considered the taste of the plant stanol ester supplement to be good or very good (68% of the responders) and they also considered the supplement to be easy or very easy to consume daily at the advised dose for four weeks (78% of the responders).

3.2. Serum and lipoprotein lipids

LDL cholesterol was reduced by 7.6% in the plant stanol group compared to controls ($P = 0.001$) (Table 3). Serum total cholesterol concentration was lowered by 4.9% and non-HDL cholesterol by 6.6% compared to controls ($P < 0.003$ for both). No statistically significant change in HDL cholesterol (mean difference −0.01 mmol/L) or serum triacylglycerols (mean difference 0.002 mmol/L) was measured between the groups.

3.3. Dietary intake

At baseline and at the end of the study (Table 4), the dietary intakes did not differ between the study groups. There was no sig-

Table 4
Dietary intake at the end of the intervention.

Variables	Plant stanol ester group, (n = 47)		Control group (n = 51)		Mean difference		P ^a
	Mean	(SE)	Mean	(SE)	Mean	(SE)	
Energy (MJ/d)	7.9	(±0.3)	7.9	(±0.3)	+0.03	(±0.5)	0.952
Protein (% of energy)	17.9	(±0.4)	18.3	(±0.5)	−0.4	(±0.7)	0.567
Carbohydrate (% of energy)	41.3	(±1.1)	39.3	(±0.9)	+1.9	(±1.4)	0.173
Fat (% of energy)	36.1	(±0.9)	36.9	(±0.9)	−0.8	(±1.2)	0.507
SFA (% of energy)	12.9	(±0.5)	12.5	(±0.4)	+0.4	(±0.6)	0.520
MUFA (% of energy)	13.6	(±0.5)	13.7	(±0.5)	−0.1	(±0.7)	0.887
PUFA (% of energy)	6.8	(±0.3)	6.7	(±0.3)	+0.05	(±0.4)	0.912
Alcohol (% of energy)	1.8	(±0.5)	2.4	(±0.6)	−0.5	(±0.8)	0.501
Cholesterol (mg/d)	240	(±23)	228	(±15)	+12.1	(±27)	0.649
Fiber (g/d)	22.1	(±1.2)	22.9	(±1.2)	−0.8	(±1.7)	0.621

^a Independent samples *t*-test.

nificant change in the nutrient intake from the baseline in either of the groups (NS for all, data not shown).

4. Discussion

The novelty of this study was the effective LDL cholesterol lowering of a new type of a small-volume low-fat plant stanol ester food supplement. In the moderately hypercholesterolemic study population, LDL cholesterol concentration was significantly reduced on average by 7.6% compared with controls, and the respective significant reductions for serum total and non-HDL cholesterol were 4.9% and 6.6%. As expected, HDL cholesterol and serum triacylglycerol concentrations were unchanged. No side effects were reported. Information was also gathered by the study population related to the sensory properties and experiences on using the supplement. The majority of the participants considered this new type chewable supplement tasty and easy to consume daily.

The design of the study was a randomized, double blind, controlled clinical intervention, and according to the power calculation, the study population was large enough to adequately address the research question. The randomization was successful regarding gender, age, weight, BMI, distribution of diseases and medication, and serum and lipoprotein cholesterol concentrations. The higher plasma glucose and serum triacylglycerol values in the control than in the plant stanol ester group did not affect the lipid results.

In general, based on recent large meta-analyses (Demonty et al., 2009; Musa-Veloso et al., 2011; Ras et al., 2014) it is considered that plant stanols and plant sterols at the dose of 2 g/day lower LDL cholesterol on average by 9–10%. Most of the studies included in these analyses were randomized controlled trials and were performed using solid food matrices with a markedly larger matrix volume than is provided in the present study. At the moment there is conflicting evidence of the efficacy of plant stanol or plant sterol esters added in low-fat and small-size delivery forms of e.g., less than 5 g such as softgel capsules (Maki et al., 2012; Maki et al., 2013; McKenney et al., 2014; Ottestad et al., 2013; Woodgate et al., 2006). In one of these studies the capsules had no LDL cholesterol lowering effect (Ottestad et al., 2013), and in two studies the efficacy was moderate, less than −5% (Maki et al., 2012; McKenney et al., 2014). Accordingly, when new types of products with added plant stanols/sterols are developed, their cholesterol-lowering efficacy should be clinically evaluated.

In order to displace cholesterol from mixed micelles, plant stanol esters must be hydrolysed in the upper part of the small intestine. The first critical step in this process is a release of the plant stanol ester from the food or food supplement matrix and mixing it with the emulsified fat in the stomach. For effective hydrolysis

of the plant stanol ester that is contained in the emulsified fat droplets, the release of bile and pancreatic enzymes must be induced. In the upper part of the small intestine pancreatic cholesterol esterase hydrolyses the ester bonds of plant stanol esters to form free plant stanols that displace cholesterol from the mixed micelles. The amount of free plant stanols has to be large enough for effective cholesterol displacement.

In the present chewable supplement the plant stanol esters were emulsified in a gelled water matrix that release the emulsified plant stanol esters effectively in the stomach. The supplement was consumed as part of a meal to ensure effective hydrolysis of the plant stanol esters. Two recent studies demonstrate the importance of consuming plant stanol or plant sterol esters even in solid food as biscuits or as a large single yoghurt drink of 100 g with a meal (Doornbos, Meynen, Duchateau, van der Knaap, & Trautwein, 2006; Kriengsinyos, Wangtong, & Komindr, 2015). When plant stanol ester biscuits were consumed in-between meals, LDL cholesterol concentration was practically unchanged, whereas when consumed with a meal, LDL cholesterol concentration was significantly reduced compared to controls (Kriengsinyos, Wangtong, & Komindr, 2015). The yoghurt drink lowered LDL cholesterol concentration when taken without meal, but the effect was significantly weaker compared to the same dose consumed with a meal (Doornbos et al., 2006). Interestingly, in the negative softgel capsule study (Ottestad et al., 2013) the unresponsiveness could not be explained by inadequate timing of consumption, since the capsules were consumed with meals.

In conclusion, the present results demonstrated that plant stanol esters in a new chewable small-volume low-fat format effectively and without side-effects lowered LDL, serum total, and non-HDL cholesterol concentrations without affecting HDL cholesterol and serum triacylglycerol levels in a moderately hypercholesterolemic study population. The majority of the participants considered the chewable supplement tasty and easy to consume daily.

Conflict of interest

At the time of clinical trial study execution, KL acted as a part-time clinical research manager at Raisio Nutrition Ltd., Raisio, Finland, which produces, licenses and markets plant stanol ester. Other authors (LK, MJN, HG, and PS) declare that they have no conflict of interest.

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Authorship

KL, LK, MJN, HG, and PS participated in the conception and design of the study. LK, HG and PS made substantial contribution to the acquisition of clinical data. KL, HG and PS contributed in analyses and interpretation of the data and in drafting and writing the manuscript. KL, LK, MJN, HG, and PS critically revised the manuscript, and KL, LK, MJN, HG, and PS approved the final version to be submitted.

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References

- Armstrong, M. J., & Carey, M. C. (1987). Thermodynamic and molecular determinants of sterol solubilities in bile salt micelles. *Journal of Lipid Research*, 28, 1144–1155.
- Davidson, M. H., Maki, K. C., Umporowicz, D. M., Ingram, K. A., Dicklin, M. R., Schaefer, E., ... Franke, W. C. (2001). Safety and tolerability of esterified phytosterols administered in reduced-fat spread and salad dressing to healthy adult men and women. *Journal of American College of Nutrition*, 20, 307–319.
- Demonty, I., Ras, R. T., van der Knaap, H. C. M., Meijer, L., Zock, P. L., Geleijnse, J. M., & Trautwein, E. A. (2009). Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. *Journal of Nutrition*, 139, 271–284.
- Denke, M. A. (1995). Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. *American Journal of Clinical Nutrition*, 61, 392–396.
- Doornbos, A. M. E., Meynen, E. M., Duchateau, G. S. M. J. E., van der Knaap, H. C. M., & Trautwein, E. A. (2006). Intake occasion affects the serum cholesterol lowering of a plant sterol-enriched single-dose yoghurt drink in mildly hypercholesterolaemic subjects. *European Journal of Clinical Nutrition*, 60, 325–333.
- Ikeda, I., Tanaka, K., Sugano, M., Vahouny, G. V., & Gallo, L. L. (1988). Inhibition of cholesterol absorption in rats by plant sterols. *Journal of Lipid Research*, 29, 1573–1582.
- Kriengsinyos, W., Wangtong, A., & Komindr, S. (2015). Serum cholesterol reduction efficacy of biscuits with added plant stanol ester. *Cholesterol*. <http://dx.doi.org/10.1155/2015/353164>. Article ID 353164.
- LaRosa, J. C. (2007). Low-density lipoprotein cholesterol reduction: The end is more important than the means. *American Journal of Cardiology*, 100, 240–242.
- Maki, K. C., Lawless, A. L., Reeves, M. S., Dicklin, M. R., Jenks, B. H., Shneyvas, E., & Brooks, J. R. (2012). Lipid-altering effects of a dietary supplement tablet containing free plant sterols and stanols in men and women with primary hypercholesterolaemia: A randomized, placebo-controlled crossover trial. *International Journal of Food Sciences and Nutrition*, 63, 476–482.
- Maki, K. C., Lawless, A. L., Reeves, M. S., Kelley, K. M., Dicklin, M. R., Jenks, B. H., Shneyvas, E., & Brooks, J. R. (2013). Lipid effects of a dietary supplement softgel capsule containing plant sterols/stanols in primary hypercholesterolemia. *Nutrition*, 29, 96–100.
- McKenney, J. M., Jenks, B. H., Shneyvas, E., Brooks, J. R., Shenoy, S. F., & Maki, K. C. (2014). A softgel dietary supplement containing esterified plant sterols and stanols improves the blood lipid profile of adults with primary hypercholesterolemia: A randomized, double-blind, placebo-controlled replication study. *Journal of the Academy of Nutrition and Dietetics*, 114, 244–249.
- Miettinen, T. A., Vuoristo, M., Nissinen, M., Järvinen, H. J., & Gylling, H. (2000). Serum, biliary, and fecal cholesterol and plant sterols in colectomized patients before and during consumption of stanol ester margarine. *American Journal of Clinical Nutrition*, 71, 1095–1102.
- Musa-Veloso, K., Poon, T. H., Elliot, J. A., & Chung, C. (2011). A comparison of the LDL-cholesterol lowering efficacy of plant stanols and plant sterols over a continuous dose range: Results of a meta-analysis of randomized, placebo-controlled trials. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 85, 9–28.
- Ottestad, I., Ose, L., Wenersberg, M. H., Granlund, L., Kirkhus, B., & Retterstøl, K. (2013). Phytosterol capsules and serum cholesterol in hypercholesterolemia: A randomized controlled trial. *Atherosclerosis*, 228, 421–425.
- Racette, S. B., Lin, X., Lefevre, M., Anderson Speare, C., Most, M. M., Ma, L., & Ostlund, R. E. Jr. (2010). Dose effects of dietary phytosterols on cholesterol metabolism: A controlled feeding study. *American Journal of Clinical Nutrition*, 91, 32–38.
- Ras, R. T., Geleijnse, J. M., & Trautwein, E. A. (2014). LDL-cholesterol-lowering effect of plant sterols and stanols across different dose ranges: A meta-analysis of randomised controlled studies. *British Journal of Nutrition*, 112, 214–219.
- Woodgate, D., Chan, C. H. M., & Conquer, J. A. (2006). Cholesterol-lowering ability of a phytostanol softgel supplement in adults with mild to moderate hypercholesterolemia. *Lipids*, 41, 127–132.